REMARKS

In response to the Non-Final Office Action dated April 1, 2009, claims 1-3 and 43 have been amended. No claims have been cancelled and no new claims have been added. It is urged that support for all the above amendments may be found throughout the specification as originally filed. No new matter has been added. The above amendments are not to be construed as acquiescence with regard to the Examiner's rejections and are made without prejudice to prosecution of any subject matter removed or modified by this amendment in a related divisional, continuation or continuation-in-part application. Following the amendments, claims 1-6, 8, 10, 13, 15, 21-25, and 43 are pending in the application. Favorable reconsideration of the subject application is respectfully requested in view of the following remarks.

Rejections under 35 U.S.C. §112, first paragraph, written description

Claim 1-6, 8, 10, 13, 15, 25, and 43 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to satisfy the written description requirement. Specifically, the Action alleges that the claimed subject matter was not described in such a way as to convey to the skilled artisan that the inventor was in possession of the claimed invention at the time of filing of the instant application.

Applicants respectfully traverse this basis of rejection.

Applicants respectfully submit that one having skill in the art would reasonably believe Applicants to be in possession of the presently claimed genus of sphingosine kinases at the time the application was filed.

The Action alleges that the as-filed specification does not describe the structure or functional nature of the numerous nucleic acid fragments or homologs thereof encoding a sphingosine kinase that will modulate one or more mammalian cell characteristics *in vivo*. Applicants respectfully disagree,

What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d at 1384, 231

USPQ at 94. >See also *Capon v. Eshhar*, 418 F.3d 1349, 1357, 76 USPQ2d 1078, 1085 (Fed. Cir. 2005) ("The 'written description' requirement must be applied in the context of the particular invention and the state of the knowledge. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution."). See MPEP § 2163(II)(A)(3)(a).

Applicants respectfully submit that sphingosine kinase sequences, fragments, and homologs thereof were amply described in the art at the time the instant application was filed. For example, U.S. Patent No. 6,730,480 ("the `480 patent"), with a priority date of May 13, 1999, discloses human sphingosine kinase-1 polypeptides, polynucleotides, and assays for measuring sphingosine kinase-1 activity. The first issued claim in `480 patent recites:

> 1. An isolated polypeptide comprising an amino acid sequence at least 90% identical to the sequence of SEQ ID NO: 2 [human sphingosine kinase-1] wherein said polypeptide has sphingosine kinase activity.

Thus, Applicants were in possession of all sphingosine kinases disclosed and claimed in the `480 patent at the time the instant application was filed.

In addition, U.S. Patent No. 7,112,427 ("the `427 patent"), with a priority date of May 13, 1999, discloses human sphingosine kinase-1 polypeptides, polynucleotides, and assays for measuring sphingosine kinase-1 activity. The first issued claim in `427 patent recites:

> 1. An isolated polynucleotide encoding a sphingosine kinase, the polynucleotide comprising (1) the sequence of SEQ ID NO:1 [human sphingosine kinase-1], (2) a sequence at least 90% identical to SEQ ID NO:1, (3) a nucleotide sequence that hybridizes to the nucleotide sequence of (1) or (2) under high stringency conditions of about 65.degree. C. and about 50% v/v formamide and about 0.15M salt, (4) a nucleotide sequence encoding a polypeptide having the sequence of SEQ ID NO:2 [human sphingosine kinase-1], or (5) a nucleotide sequence complementary to the nucleotide sequence of any one of (1) to (4).

Thus, Applicants were in possession of all sphingosine kinases disclosed and claimed in the `427 patent at the time the instant application was filed.

Furthermore, Pitson et al., *Biochem J.* Sep 1;350 Pt 2:pp. 429-41, 2000a, (of record) disclose homologs of the human sphingosine-1 kinase in mouse, rat, monkey,

S. cerevisiae, *S. pombe*, *A. thaliana*, and *O. sativa* and evolutionarily conserved sequences thereof. Kohama et al., 1998 (of record) disclose two murine sphingosine kinase one splice variants, evolutionary conserved sequences compared to human, yeast, and nematode sphingosine-1 kinases, and sphingosine kinase activity assays (see, for example Figures 5 and 6 of Kohama). Pitson et al., 2002 (of record) disclose no less than 14 human sphingosine kinase-1 mutants that have reduced sphingosine kinase activity (see, for example Figure 3 and Table 1). Pitson et al. (*The Journal of Biological Chemistry*. Vol. 275, No. 43, pp. 33945–33950, 2000b; copy attached) disclose site-directed mutagenesis of Gly82 to Asp (G82D) of the human sphingosine kinase-1. The residue was identified through sequence conservation between several diacylglycerol kinases and sphingosine kinases (see, for example, Figure 1). The G82D mutation acts as a dominant negative to block activation of sphingosine kinase-1 activity and sphingosine kinase-1 cell signaling. In addition, the abstract of Pitson et al. (*FEBS Lett.* 2001 Dec 7; 509(2):169-73; abstract attached) discloses a human sphingosine kinase 1 point mutant, Gly113 to Ala (G113A), with increased catalytic activity. The residue was identified through sequence conservation.

Accordingly, Applicants respectfully submit that in view of the knowledge in the art, Applicants were in possession of the presently claimed sphingosine kinases.

The Action further alleges that the specification is silent on the specific characteristics, or sequence motifs of sphingosine kinase or homolog thereof that may contribute to a therapeutic treatment and/or prophylaxis. Applicants respectfully disagree.

Applicants submit that the claims are directed to methods of modulating one or more mammalian endothelial cell functional characteristics, the method comprising inducing over-expression of sphingosine kinase, functional fragment thereof, or homolog thereof having sphingosine kinase activity. One having skill in the art would appreciate that the claims clearly recite sphingosine kinases having sphingosine kinase activity which is an important aspect of the therapeutic treatment and/or prophylaxis (see, for example, Figures 11, 15, and 16 and Example 2).

Further, the Action alleges that the specification does not provide any teachings whether such fragment would retain the function of sphingosine kinase and that there is no identification of any particular portion of the structure that must be conserved. Applicants respectfully disagree.

As noted above, Pitson et al., 2001 and Pitson et al., 2001 provide examples of highly conserved regions in sphingosine kinase type I kinases that were mutated to either inhibit or increase sphingosine kinase activity. One having skill in the art would appreciate that this is a standard method in the art, as sequences conserved across evolution are thought to be functionally important sequences. Thus, Applicants submit that it is the highly conserved regions that inform where functional portions of the molecule are located and where mutations could be made.

Accordingly, the U.S.P.T.O. believes Applicants to be in possession of a considerably large genus of sphingosine kinase-1 molecules (see, U.S. Patent Nos. 6,730,480 and 7,112,427) and the art has provided examples of homologs, functional fragments, and methods of identifying the same (see, for example Kohama et al., 1998, Pitson et al., 2000a, Pitson et al., 2000b, and Pitson et al., 2001). Thus, one having skill in the art would reasonably conclude that Applicants were in possession of the presently claimed genus of sphingosine kinases at the time the application was filed.

Thus, Applicants respectfully submit that the as-filed specification comports with the written description requirement of § 112. Reconsideration and withdrawal of this basis for rejection is respectfully requested.

Rejections under 35 U.S.C. §112, first paragraph, enablement

Claims 1-6, 8, 10, 13, 15, 25, and 43 stand rejected under 35 U.S.C. §112, first paragraph, because the specification allegedly does not reasonably enable the modulation of one or more mammalian endothelial cell functional characteristics by way of the claimed methods *in vivo*. However, the Action acknowledges that the specification is enabled for overexpression of

a nucleic acid encoding sphingosine kinase introduced into mammalian endothelial cells that results in enhancing cell survival, altering adhesion molecule expression, enhancing neutrophil adhesion to endothelial cells, promoting tube formation or formation of a capillary network of endothelial cells *in vitro*.

Specifically, the Action alleges that the specification fails to provide any relevant teachings or specific guidance or working examples with regard to the production of sphingosine kinase *in vivo*, by modulating the functional level of sphingosine kinase in a mammal resulting in the treatment and/or prophylaxis of a condition characterized by aberrant or otherwise unwanted endothelial cell function. Therefore, the Examiner concludes that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate with the scope with the claims.

Applicants respectfully traverse this basis of rejection.

Applicants respectfully submit that the presently claimed invention is fully enabled by the as-filed specification. Moreover, Applicants respectfully submit that the skilled artisan would not encounter any undue experimentation in practicing the full scope of the presently claimed invention.

As an initial point, Applicants respectfully note that claims 1-3 and 43 have been amended, without acquiescence and solely to clarify a particular aspect the presently claimed invention, to recite wherein the sphingosine kinase is introduced into an endothelial cell. Antecedent basis for the term "endothelial cell" is provided in the preamble of claims 1-3 and throughout the specification as-filed. Thus, no new matter has been introduced by way of these amendments.

The Action alleges that the claims embrace introducing a sphingosine kinase nucleic acid in any type of cell *in vivo* in order to modulate endothelial cell characteristics or treat and/or provide prophylaxis for a condition characterize by unwanted endothelial cell function. In contrast to the Action's allegations, the presently claimed methods are directed to the overexpression of sphingosine kinase in endothelial cells and not the overexpression of sphingosine kinase in any cell.

The Action further alleges that the presently claimed methods fall into the realm of gene therapy and that gene therapy was unpredictable and required undue experimentation at the time the application was filed. The Action further contends that the as-filed specification does not provide any relevant teachings, specific guidance, or working examples for overcoming the limitations of sphingosine kinase gene transfer *in vivo* that would result in the modulation of endothelial cells *in vivo* or in the treatment and/or prophylaxis of a disease. Applicants respectfully disagree and point out that the as-filed specification provides ample teachings, specific guidance, or working examples to practice the presently claimed invention. Moreover, no working examples are required to satisfy the enablement requirement.

Applicants respectfully submit that compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, does not turn on whether an example is disclosed. An example may be "working" or "prophetic." An applicant need not have actually reduced the invention to practice prior to filing. *In Gould v. Quigg*, 822 F.2d 1074, 1078, 3 USPQ 2d 1302, 1304 (Fed. Cir. 1987). The specification need not contain an example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904, 908, 164 USPQ 642, 645 (CCPA 1970). See MPEP § 2164.02.

However, in contrast to the allegations raised in the Office Action, the as-filed specification provides many working examples of the presently claimed invention. Applicants respectfully submit that the as-filed specification provides ample guidance for the expression of a sphingosine kinase in endothelial cells. For example, the as-filed specification teaches that overexpression of sphingosine kinase using an adenoviral vector *in vitro* leads to increased endothelial cell survival in adenoviral treated cells compared to control cells (see Example 1 of the as-filed specification). Furthermore, endothelial cells treated *in vitro* with adenoviral sphingosine kinase display lower levels of caspase-3 activity compared to control cells (see Example 1 of the as-filed specification). Example 2 of the as-filed specification shows that endothelial cells treated *in vitro* with adenoviral sphingosine kinase display increased vascular cell adhesion molecule expression, increased neutrophil adhesion, and vascular tube formation.

Applicants respectfully submit that these *in vitro* examples correlate with the presently claimed methods. Applicants reiterate that the use of *in vitro* experiments to establish *in vivo* events is, in principle, a valid methodology. *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 U.S.P.Q. 739 (Fed. Cir. 1985); *Nelson v. Bowler*, 626 F.2d 853, 856, 206 U.S.P.Q. 881 (C.C.P.A. 1980). In fact, the MPEP § 2164.02, states that an *in vitro* or *in vivo* animal model example in the specification, in effect, constitutes a "working example" if that example "correlates" with a disclosed or claimed method invention. Furthermore, the Examiner must weigh the evidence for and against correlation and decide whether one skilled in the art would accept the model as reasonably correlating to the condition. *In re Brana*, 51 F.3d 1560, 1566, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995) (reversing the PTO decision based on finding that *in vitro* data did not support *in vivo* applications). Since the initial burden is on the Examiner to give reasons for the lack of enablement, the Examiner must also give reasons for a conclusion of lack of correlation for an *in vitro* or *in vivo* animal model example. A rigorous or an invariable exact correlation is not required, as stated in *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 USPQ 739, 747 (Fed. Cir. 1985).

In the instant case, the Action alleges that the presently claimed methods fall into the realm of gene therapy and that gene therapy was unpredictable and required undue experimentation at the time the application was filed. The Action further alleges that the delivery of a nucleic acid to tissue culture cells does not provide guidance for overcoming the obstacles of *in vivo* delivery, because the nucleic acid does not have to cross through the complex organization of organs and tissues. The Action further contends that *in vitro* studies of a protein's function at the cellular level is problematic due to interactions with other molecules and precludes the studies of physiological (*e.g.*, metabolic pathways) and phenotypic functions in a mammal (*e.g.*, role of the protein in the whole mammal).

Applicants respectfully submit that there are no three dimensional structures, blood vessels, or connective tissues through which the nucleic acid would be required to cross through *in vivo* to effect therapy. Adenovirus is routinely administered via the vasculature which contacts all endothelial cells. In fact, some types of endothelial cells are part of the vasculature

(*e.g.*, vascular endothelial cells). The art of gene therapy at the time the application was filed had successfully solved the problem of delivering adenoviral constructs to endothelial cells. Applicants respectfully submit herewith, examples of adenoviral administration to endothelial cells, wherein *in vitro* cell culture models correlate to the *in vivo* models. See, for example, Lemarchand et al. *Circulation Research* 1993, 72, 1132-1138; Schulick et al. *Circulation Research* 1995, 77, 475-485; Budenz et al. *Investigative Ophthalmology and Visual Research*, 1995, 36, 11, 2211-2215; Zoldhelyi et al. *Circulation* 1996, 93, 10-17; White et al. *Hypertension* 2001, 37, 449-455; Champion et al. *Circulation Research*, 1999, 84, 1422-1432; and Claudio et al. *Circulation Research* 1999, 85, 1032-1039; a copy of each reference is attached for your convenience.

Moreover, Applicants respectfully submit that Duan et al., 2007, previously made of record, provides post-filing examples of the reduction to practice of adenoviral sphingosine kinase administration in mammals. In addition, the reference correlates *in vitro* observations to support their studies. In fact, Duan et al. expressed sphingosine adenovirus in isolated rat cardiac myocytes, isolated rat hearts, and *in vivo* rat hearts. However, the Examiner objected to Duan because the effects allegedly involved cardiac muscle cells and not endothelial cells. Applicants respectfully submit that Duan et al., applies to the principle that *in vitro* models of adenoviral shpingosine kinase reasonably correlate with *in vivo* models. To further bolster this point, Applicants submit a post-filing example, Lee et al., *Coronary Artery Disease*, 2005, 16, 451-456, (copy attached) which describes the use of adenoviral sphingosine kinase to promote arteriogenesis in a rabbit hindlimb ischemia model. Lee et al. provide a correlation of an *in vitro* endothelial cell model with an *in vivo* endothelial cell model using sphingosine kinase, as disclosed in the as-filed specification.

The Action further contends that *in vitro* studies of a protein's function at the cellular level is problematic due to interactions with other molecules and precludes the studies of physiological (*e.g.*, metabolic pathways) and phenotypic functions in a mammal (*e.g.*, role of the protein in the whole mammal). Applicants fail to understand the Examiner's rationale. Such rationale would preclude the correlation of any *in vitro* assay to an *in vivo* assay, unless the assay

environments were indistinguishable. Applicants respectfully submit that this rationale is not only impractical, but irrelevant.

Applicants further submit that the skill in the art of adenoviral therapy is high and that one having skill in the art would not encounter undue experimentation in delivering an adenovirus to a given treatment site. If a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 U.S.C. § 112 is satisfied. *In re Johnson*, 282 F.2d 370, 373, 127 USPQ 216, 219 (CCPA 1960); *In re Hitchings*, 342 F.2d 80, 87, 144 USPQ 637, 643 (CCPA 1965). See also *In re Brana*, 51 F.2d 1560, 1566, 34 USPQ2d 1437, 1441 (Fed. Cir. 1993). See MPEP § 2164.01(c). For example, it is not necessary to specify the dosage or method of use if it is known to one skilled in the art that such information could be obtained without undue experimentation. See MPEP § 2164.01(c).

The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *In re Certain Limited-Charge Cell Culture Microcarriers,* 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983), aff 'd. subnom., *Massachusetts Institute of Technology v. A.B. Fortia,* 774 F.2d 1104, 227 USPQ 428 (Fed. Cir.1985). See also *In re Wands,* 858 F.2d at 737, 8 USPQ2d at 1404. The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976).

Applicants respectfully submit that the guidance provided by the as-filed specification in combination with the knowledge and level of skill of one in the art would enable the skilled artisan to practice the presently claimed inventions without undue experimentation. Moreover, the skilled artisan would conclude that the *in vitro* experiments provided in the as-filed specification reasonable correlate to the presently claimed methods. Thus, as the Action has failed to provide any evidence that an adenovirus encoding a sphingosine kinase used in an *in vitro* endothelial cell culture model would not reasonable correlate with an *in vivo* application of the model, the Action has failed to meet its burden to establish lack of enablement.

Accordingly, Applicants respectfully submit that the as-filed specification provides ample guidance to enable one having skill in the art to practice the overexpression of an isolated nucleic acid molecule encoding sphingosine kinase *in vitro* and *in vivo* to modulate endothelial functional cell characteristics, or functional fragment or homolog thereof, wherein said kinase, functional fragment thereof, or homolog thereof comprises sphingosine kinase activity, without undue experimentation. Reconsideration and withdrawal of this basis for rejection is respectfully requested.

Double patenting rejections

Claims 1-3, and 5 stand rejected for non-statutory obviousness-type double patenting as allegedly being unpatentable over claims 1-6, 15-20 of U.S. Patent No. 10/275,686.

Further, claims 1-2, 5-7, and 15 stand rejected for non-statutory obviousness type double patenting as allegedly being unpatentable over claims 1-15, 17, and 23 of U.S. Patent No. 09/977,217.

Applicants respectfully traverse this rejection and submit that the pending claims have not issued and the Examiner has not indicated that they are allowable, and thus, the present claims may be considerably amended during prosecution. As the 10/275,686 and 09/977,217 are presently owned by the same entity of the present application, Applicants request that this rejection be withdrawn in this application with the understanding that Applicants will file a Terminal Disclaimer, if appropriate, in the instant application. Until such time as the present claims are in condition for allowance, Applicants respectfully submit that the filing of a terminal disclaimer is premature.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

All of the claims remaining in the application are now believed to be allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

SEED Intellectual Property Law Group PLLC

/Michael J. McDonald/
Michael J. McDonald, Ph.D., Patent Agent
Registration No. 62,581

MJM/lhk

Enclosures: As noted

701 Fifth Avenue, Suite 5400
Seattle, Washington 98104
Phone: (206) 622-4900
Fax: (206) 682-6031

1475337_1.DOC